

Some Relations Between the Chemical and Physical Characteristics of Bovine Muscles

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SUMMARY

Variations in the distribution of water in bovine muscles were investigated with histological and chemical analyses. A histological method has been developed to measure the extracellular space. This property, along with water and protein content and pH values, was determined in four muscles from each of four animals. The results indicate that extracellular space varied among the muscles of the animals according to a definite pattern. Among the relations found, extracellular space was positively correlated with the water-protein ratio, whereas intracellular water content was negatively correlated. The evidence indicates that, in the muscles that characteristically contain a relatively high proportion of water to protein, the additional water is located in extracellular spaces.

Recent investigations in this laboratory have been concerned with the properties of bovine muscle tissues related to their capacity for holding water (Swift and Berman, 1959; Swift *et al.*, 1960; Berman, 1961; Gibbs and Fryar, 1961). Patterns established for the composition and certain properties of the muscles have been found to repeat in the muscles of each different animal studied. Among the components conforming to pattern and having a direct statistically significant relation to holding water are the chloride content and water-protein ratio.

From the chloride content is calculated "chloride space," a measure of the extracellular space in which aqueous fluid is found. The variation in chloride content therefore suggested that differences may exist in the distribution of water within the muscles. The work reported here deals with an investigation of this possibility.

The measurement of differences in extracellular areas is a direct approach to obtaining information on variations in chloride space and water-protein ratios. A satisfactory histochemical method was needed as an independent and direct approach to this measurement. To accomplish this, histo-

logical techniques that minimize distortion were investigated and devised.

The newly developed method and complementary chemical methods were then applied to a group of four muscles from each of four bovine animals. The muscles selected were those that, in our earlier work, had been found to vary markedly in composition and properties. The present paper describes our new histochemical method, presents the histochemical and chemical data obtained, and discusses the significance of the knowledge obtained on the distribution of water among the muscles.

EXPERIMENTAL PROCEDURE AND RESULTS

Animals and muscles used. The carcasses used were of three Holstein animals (a cow 34 months old, a bull 15 months old, and a bull 6 months old) and a Brown Swiss cow (39 months old). Within one hour of slaughter the muscles listed in Table 1 were removed from each carcass. Centrally located cubes (2 cm³) were cut from each muscle for histological analysis, and adjacent cubes for chemical analysis.

Histological method. The cubes to be used for histological analysis were immediately frozen by immersion in liquid nitrogen (-195°C) contained in DeWar flasks. The cubes were then transferred to solid CO₂ (-78°C) and stored at dry-ice tem-

Table 1. Bovine muscle studied

Number	Muscle	Location	
		Forequarter	Hindquarter
1	<i>Longissimus dorsi</i>	Rib, chuck	Sirloin, porterhouse
2	<i>Semimembranosus</i>		Round
3	<i>Serratus ventralis</i>	Rib, chuck	
4	<i>Rectus abdominus</i>	Plate	Flank

perature until sectioned (two or more hours later).

The sample (stored at -78°C) was removed from storage and allowed to warm until microtome cutting temperature was reached. The 2-cm³ sample was quickly cut to 1-cm³ size and attached to the stage of a Spencer sliding microtome (trade names are mentioned for identification, implying no endorsement) with a drop of water that was then frozen in a stream of CO₂ gas. Cross sections of the muscle were cut 15 μ thick and mounted with glycerol directly on alcohol-cleaned slides. The sections were stained lightly according to Lillie's (1942) modification of Mayer's hematoxylin method and blotted with filter paper. A drop of glycerol was placed on the slide, and the section was covered with a 2-mm No. 0 micro cover glass.

By using liquid nitrogen and solid CO₂, this method avoided fixation procedures. Flotation was also eliminated by direct mounting with glycerol. This treatment resulted in minimal distortion of the muscle sections.

The mounted sections were photographed with a microscope and camera, using 35-mm high-contrast copy film. To measure the magnification factor, a stage micrometer slide marked in .01-mm units was photographed with each group of slides, using the same setting of microscope and camera. Enlarged 5 \times 8-in. prints were made of muscle cross sections and of the stage micrometer with the same setting of the enlarger. In this way, accurate measurements of the original cell dimensions were possible.

The cellular and extracellular areas were cut out of the photograph, the respective areas of the paper were weighed, and the percentage of extracellular space was calculated from the weights. Calculation of space, a volume, from area, a plane surface, was possible because of the geometry of muscle tissue and the technique of sectioning tissue. Muscles are composed of bundles of fibers that may be assumed to be parallel over comparatively short distances. A transverse cross section of striated muscle would thus be approximately equivalent to a number of cylindrical fibers imbedded in a bath of extracellular material. The volumes of the fiber and the non-fiber materials can be calculated by multiplying these cross-sectional areas by this length. The lengths of the fiber and non-fiber

material are equal when a sectional cut is made perpendicular to the axis of the fibers. Consequently, the volumes of the fiber and non-fiber materials are directly proportional to their areas. The average cellular diameter was calculated by dividing the total cellular area in a photograph by the number of cells, assuming the area of each cell to be a circle. The effect of post-mortem age was determined on samples from the other half of the carcass after 24 hr at *ca.* 3–4°C. In all instances histological measurements were made on 5–8 samples of tissue, and the data given are averages of these values.

Analytical methods. The cubes cut from the muscles for chemical analyses were ground through a Hamilton-Beach grinder equipped with a plate having approx 5-mm² openings. The analytical methods were applied to duplicate samples of ground tissue. Total nitrogen was measured by the macro-Kjeldahl method, and moisture by the oven drying method of the AOAC (1960). To determine pH, 20 g of each sample were mixed with 20 ml of water and stored at 5°C in airtight containers. After 24 hours, the samples were allowed to warm to room temperature and the pH was determined with a Beckman Model GS pH meter. This value has become known as the ultimate pH and corresponds to the low pH attained in tissue after rigor. Chloride was determined by the modified Volhard method described by Wilson and Ball (1929). Chloride space was calculated from the chloride content of the tissue and of blood, using the equation:

$$\text{Chloride space, \%} = \frac{\text{tissue chloride, mg \%}}{\text{blood chloride, mg \%}} \times 100.$$

Results and calculations. Table 2 shows average values obtained in the chemical analyses and histological measurements, along with the results of calculations of moisture-to-protein ratios. Fig. 1 shows averaged data for each of the four muscles of the four animals.

The correlation coefficients of relationships between components are given in Table 3. One property shown, intracellular water percent, was calculated as the difference between the percentages of total water content and of extracellular space. This calculation is based on the assumption

Table 2. Analytical data from histochemical and chemical analysis of four beef muscles from four different animals.

Animal	Muscle no.	Moisture	Protein	m/p Ratio ^a	Extracellular space	pH ^b	Chloride space ^c
		%	%		%		%
A (cow, 34 mo)	1	75.81	23.13	3.28	11.35 (18.70) ^d	5.50	
	2	75.72	22.15	3.42	14.40 (22.90)	5.39	
	3	76.10	20.41	3.73	18.39 (28.43)	5.63	
	4	75.79	22.01	3.44	15.02 (28.35)	5.51	
B (bull, 15 mo)	1	73.71	22.38	3.29	12.50	5.54	15.99
	2	73.40	22.06	3.33	12.01	5.42	18.67
	3	74.20	21.28	3.48	16.79	5.81	19.92
	4	74.45	21.31	3.49	13.62	5.69	20.76
C (bull, 6 mo)	1	75.00	22.82	3.29	15.54	5.45	
	2	75.34	22.99	3.28	14.48	5.39	
	3	75.89	19.53	3.89	19.48	5.69	
	4	75.57	20.42	3.70	18.11	5.59	
D (cow, 39 mo)	1	71.86	21.25	3.38	15.19	5.45	8.28
	2	74.25	22.10	3.36	14.27	5.40	10.85
	3	67.24	18.47	3.64	19.80	4.70	14.25
	4	72.45	20.82	3.48	16.33	5.47	12.74

^a Correlation coefficients of relation of extracellular to the water-protein ratios for individual animals are: Animal A, $0.980 \pm .019$, $P < .01$; B, $0.745 \pm .212$, not sig.; C, $0.985 \pm .013$, $P < .01$; D, $0.992 \pm .007$, $P < .01$.

^b Correlation coefficients of relation of extracellular spaces to the pH values for individual animals are: Animal A, $0.604 \pm .284$, not significant; B, $0.898 \pm .087$, $P < 0.10$; C, $0.998 \pm .002$, $P < .01$; D, $0.998 \pm .011$, $P < .01$.

^c Calculated as $\frac{\text{chloride tissue, mg \%}}{\text{chloride blood, mg \%}} \times 100$.

^d Determined 24 hr after slaughter.

Table 3. Statistical analyses of data from analyses on four muscles from each of four beef animals.

	Water-protein ratio		pH ^a		Protein, %	
	Averaged ^b df 3	Not averaged ^c df 15	Averaged ^b df 3	Not averaged ^c df 15	Averaged ^b df 3	Not averaged ^c df 15
Extracellular space, %	$0.988 \pm .011$ $P < .01$	$0.846 \pm .051$ $P < .001$	$0.958 \pm .037$ $P < .01$	$0.557 \pm .124$ $P < .05$	$-0.996 \pm .004$ $P < .001$	$-0.850 \pm .050$ $P < .001$
Intracellular water, ^d %	$-0.950 \pm .044$ $P < .02$	$-0.575 \pm .121$ $P < .01$	$-0.962 \pm .033$ $P < .01$	$-0.476 \pm .139$ $P < 0.10$	$0.972 \pm .025$ $P < .01$	$0.876 \pm .042$ $P < .001$

^a pH 24 hr after slaughter.

^b Based on averages of data for each of the four muscles.

^c Data treated as determinations of 16 unrelated muscles.

^d Calculated as the difference between content of water and extracellular space.

that extracellular space consists primarily of water and that the rest of the water is intracellular.

The statistical calculations were made on two bases. One involved averaged data for each muscle and emphasizes variations between the properties of muscles within animals, excluding between-animal differences; the other was based on individual measurements, independent of the muscles or animals from which the samples were obtained.

DISCUSSION

The histological method, developed after many attempts with alternative techniques, produced excellent slides for photography that were not detectably distorted (Fig. 2 and 3). In addition, the operations were carried out rapidly so that slides were immediately available for microscopic inspection and assessment. This opportunity to screen tissue sections before photomicrography eliminated lengthy operations on unsatisfactory sections.

Another method was also successful. This likewise involved initial freezing with liquid nitrogen at about -195°C . The frozen cube was then dehydrated in solutions of 1% HgCl_2 in absolute ethanol at temperatures ranging from -40°C to room temperature, treated successively with 4% collodion in alcohol, chloroform-alcohol, and paraffin in chloroform, and then finally embedded in paraffin, cut, and stained in the conventional manner. However, elapsed time was almost a month, in contrast with the rapid preferred method, which only took a few hours.

Since the measurement of extracellular space has frequently been approached through the measurement of the chloride content of tissue and the calculation of *chloride-space*, as discussed by Davson (1952), analyses by this method were made on two animals (Table 2, animals B and D). This afforded an opportunity for a limited comparison of the two methods. Statistical analyses of the results show that estimates of extracellular space based on the chloride method approximated those based on the histological method. However, estimates of extracellular space based on the chloride method varied more widely than those based on the histochemical method, and statistical analyses showed that data obtained by the two methods were not closely related.

As shown by the data in Table 2 (animal A), a drastic increase, amounting to an average of 66%, occurred in the extracellular spaces of the four muscles during the 23-hr period between samples taken at 1 hr and samples taken at 24 hr after slaughter. The correlation coefficient showing the relation between the measurements made at 1 hr and those made at 24 hr ($r = 0.874 \pm .113$, $p < .05$) indicates that the increases were moderately uniform. Therefore, any changes in the dimensions of the extracellular spaces in the hour that normally elapsed from time of slaughter until initial freezing would probably affect all muscles proportionally, and consequently can be eliminated as a factor significantly affecting the results reported here.

The changes in space after slaughter cannot be studied by determining chloride, sucrose, or inulin space. On the other hand, as shown above, the histological method can be used to measure changes occurring during the first 24 hr. Also, measurements (not reported here) on muscles aged 8 days at 3°C showed that structural deformation was marked during this period.

Analytical and statistical results are shown in Table 2 and the principal statistical calculations in Table 3. The data were subjected to calculations and statistical analyses in the manner previously followed in these investigations (Swift and Berman, 1959). The statistical analyses show that average extracellular spaces were significantly and positively correlated with average water-protein ratios ($r = 0.988 \pm .011$, $P < .01$) and average pH values ($r = 0.958 \pm .037$, $P < .01$) and negatively with average protein content ($r = -0.996 \pm .004$, $P < .001$). When based on analyses of individual animals (Table 2) the relations of extracellular spaces to water-protein ratios and pH values were also significant in three of four cases. The results indicate that variation of extracellular space occurs among the muscles of bovine animals according to definite patterns. As mentioned above, earlier work has shown that variation of pH, protein content, and other components and properties among muscles similarly occurs according to definite patterns, i.e., muscles of each animal, when listed in order of increasing

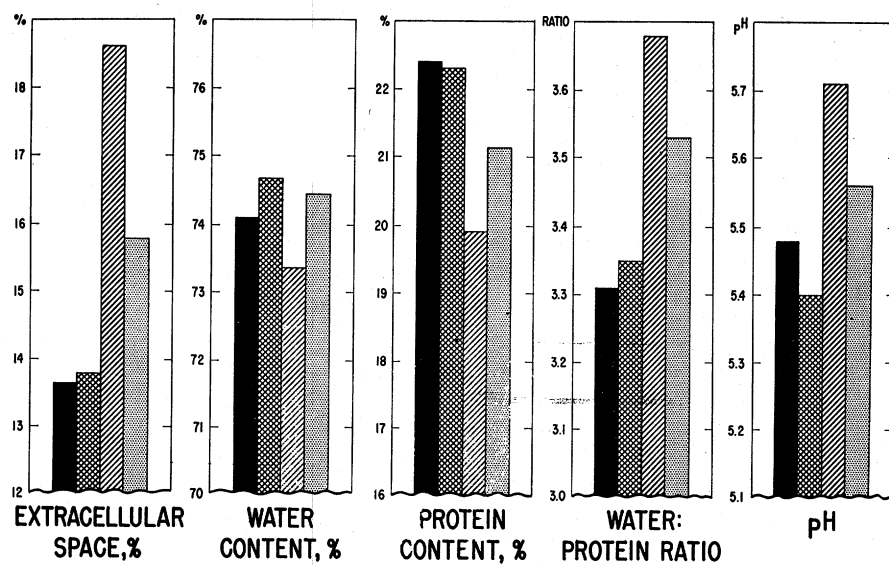
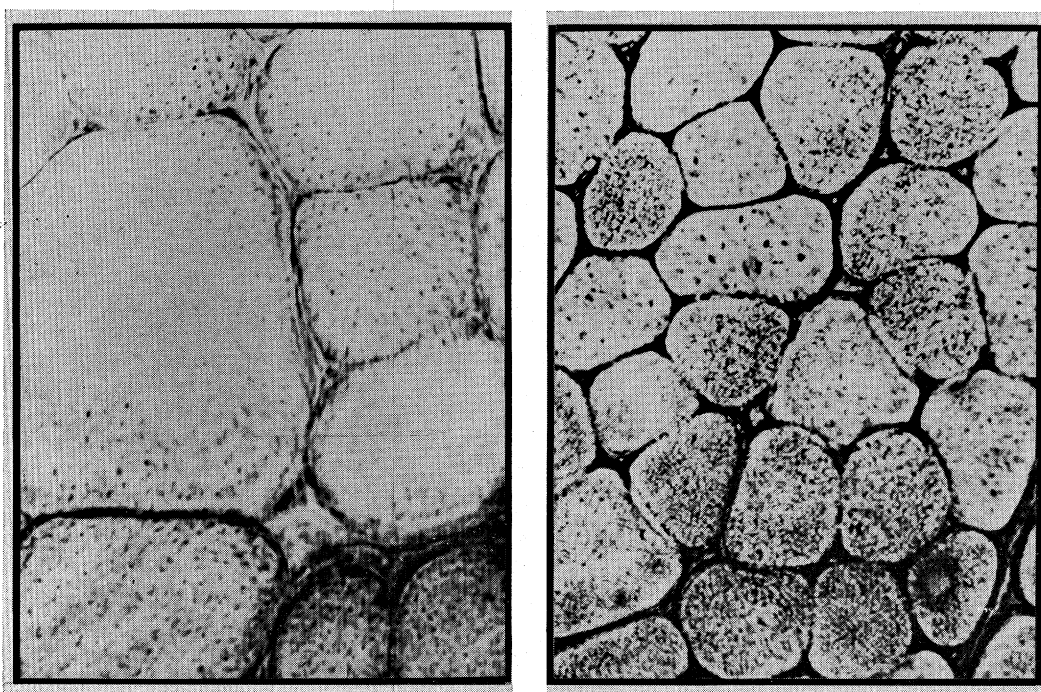


Fig. 1. Averaged data, for each of the four muscles of the four animals.



LONGISSIMUS DORSI

RECTUS ABDOMINUS

Fig. 2. Frozen sections 1 hour after slaughter, enlarged 317 \times .

amounts of extracellular space, tend to fall in the pattern $2 < 1 < 4 < 3$.

The statistical analyses in Table 3, calculated from the data on 16 muscles but not averaged or grouped according to animal or muscle sources, also show that significant correlations exist between extracellular spaces and water-protein ratios ($r = 0.846 \pm .051$, $P < .001$), pH values ($r = 0.557 \pm .124$, $P < .05$), and protein contents ($r = -0.850 \pm .050$, $P < .001$). Coefficients were lower than those obtained with averaged data, as was to be expected since calculation with the unaveraged data was influenced by between-animal differences. These arise from differences in leanness, age, sex, or conditions, which may raise or lower the levels over which the properties of all tissues may range, but do not influence patterns of between-muscle variation. The differences noted between correlation coefficients obtained in calculations based on averaged and unaveraged data derive from this fact, namely, that variations of properties and composition of muscles of animals occur in patterns that are similar but not necessarily superimposable.

The significant and direct relation of extracellular spaces to water:protein ratios shown in Table 3 indicates that the increment of water in the extracellular spaces is higher in muscles that contain a relatively high proportion of water-to-protein than in muscles that contain a lower content of water relative to protein. The *serratus ventralis* and the *rectus abdominus* muscles are those containing the high proportion of water to protein, and the *longissimus dorsi* and the *semimembranosus* the low. The validity of the interpretation depends on water being the principal component filling extracellular spaces. That the space contains fluid that is 90% water or more can be readily calculated from data on the composition of interstitial fluid published by Davson (1952).

Assuming that extracellular spaces are occupied by water allows intracellular water to be calculated. To do this, the difference between total water content and extracellular space (as water) is used as an estimate

of intracellular water. This assumption was the basis for the calculations in Table 3 of the statistical relations involving intracellular water. The significant negative relation of average percentages of intracellular water to average water-protein ratios ($r = -0.950 \pm .044$, $P < .02$) indicates that the water in the extracellular areas and not that in the cellular areas accounts for the relatively high proportion of water relative to protein in the *serratus ventralis* and *rectus abdominus* muscles.

In support of the thesis that the high water-protein ratio in the *serratus ventralis* and *rectus abdominus* muscles is entirely explained by water in their larger extracellular spaces rather than in their cellular areas, the proteins, which are principally located in the intracellular areas, appear to be hydrated at a relatively uniform level, as shown by the positive relation of percentages of intracellular water to protein content ($r = 0.972 \pm .025$, $P < .01$).

REFERENCES

- A.O.A.C. 1960. Official Methods of Analysis, 9th ed. Assoc. Official Agr. Chemists, Washington, D.C.
- Berman, M. D. 1961. Factors affecting the water retention of beef. V. Variation of the zinc-containing enzymes. *J. Food Sci.* **26**, 422.
- Davson, Hugh. 1952. "A Textbook of General Physiology." Blakiston Co., New York, N.Y.
- Gibbs, R. J., and A. J. Fryar. 1961a. Factors affecting the water retention of beef. III. Effects of pH and ionic strength. Ms. in preparation.
- Gibbs, R. J., and A. J. Fryar. 1961b. Factors affecting the water retention of beef. IV. Extractability of non-protein and protein components. Ms. in preparation.
- Lillie, R. D. 1942. An improved acid hemalum formula. *Stain Technol.* **17**, No. 2, 89.
- Swift, C. E., and M. D. Berman. 1959. Factors affecting the water retention of beef. I. Variations in composition and properties among eight muscles. *Food Technol.* **13**, 365.
- Swift, C. E., M. D. Berman, and C. Lockett. 1960. Factors affecting the water retention of beef. II. Variation in some pH determinants among eight muscles. *Food Technol.* **14**, 74.
- Wilson, D. W., and E. G. Ball. 1929. A study of the estimation of chloride in blood and serum. *J. Biol. Chem.* **79**, 221.